

Discordant Population Genetic Structuring of Smallmouth Bass, *Micropterus dolomieu* Lacepède, in Lake Erie Based on Mitochondrial DNA Sequences and Nuclear DNA Microsatellites

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ABSTRACT. The population genetic structure of smallmouth bass, *Micropterus dolomieu*, in Lake Erie was investigated using two mitochondrial DNA sequences (control region and cytochrome *b*) and eight nuclear DNA microsatellite loci. The objective was to evaluate relative resolution of fine-scale versus broad-scale spawning population genetic structure across Lake Erie. Results showed that only cytochrome *b* sequences supported the divergence of populations grouped by basins, suggesting little correspondence to bathymetric features of Lake Erie. The majority of sampling sites were characterized by large within site variances, particularly with the microsatellite data, reducing the efficiency to delimit populations with the given sample sizes. Although conclusions from mtDNA and microsatellites were not corroborative, all data sets revealed some divergent sites across and within basins. The apparently weak genetic structuring of populations does not reflect the strong behavioral patterns of male nest-site fidelity and adult migration. Finer-scale structure among geographically proximate sites was detected primarily with microsatellite data. Several locations were consistently identified as most genetically divergent suggesting that they may serve as long-term attractor areas for spawning populations. Mitochondrial DNA data indicated a broader-scale pattern reflecting either colonization from at least two glacial refugia or different dispersal routes from a common refugium with subsequent genetic divergence through drift. Genetic variation of smallmouth bass in Lake Erie is likely a product of glacial history with behavioral and stochastic factors interacting at different spatial and temporal scales. A precautionary management approach would weigh both genetic and behavioral patterns and develop appropriate conservation strategies for a non-panmictic smallmouth bass population in Lake Erie.

INDEX WORDS: Control region, cytochrome *b*, Lake Erie, *Micropterus dolomieu*, microsatellites, population genetics.

INTRODUCTION

Despite the relative economic importance of the smallmouth bass (*Micropterus dolomieu* Lacepède) and its ecological position as a top predator, few studies have investigated its population genetics (e.g., Stark and Echelle 1998) and none within a large lake system. Studies of smallmouth bass have

focused on ecological effects of stocking and introgression (e.g., Snyder *et al.* 1996), fitness analysis (e.g., Gross *et al.* 1994, Gross and Kapuscinski 1997), nesting success, survival of early life history stages, recruitment, and growth rates (e.g., Steinhart *et al.* 2004a, b). The present genetic analysis of smallmouth bass in Lake Erie complements these ecologically focused studies. Further, an integrative approach of ecology and genetics will facilitate de-

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velopment and implementation of sustainable and effective conservation policies for user groups—particularly in the face of increasing habitat degradation, fishery exploitation, and the alteration of native community interactions by invasive species.

Understanding how and why biological populations are partitioned is essential for conservation goals. Population genetic structure can result from a variety of factors (Bossart and Prowell 1998) including historical, ecological, and stochastic events that interact with varying intensities (Haydon *et al.* 1994), but ultimately must be interpreted within a life history framework (Waples 1998). Genetic structure is most robustly interpreted using a combination of different molecular markers (Ward and Grewe 1994). Thus a primary goal of this research is to investigate the population genetics of smallmouth bass in Lake Erie using two regions of the mitogenome (control region, cytochrome b) and eight nDNA microsatellite loci. These empirical results are compared with direct evidence of population demographics and structure. Our specific objectives are (1) to describe the genetic variability present in Lake Erie smallmouth bass, (2) to uncover any pattern of genetic structure, (3) to explore possible relationships between genetic variation and its geographic distribution, and finally (4) to evaluate genetically based conclusions within an ecologically based framework of smallmouth bass.

Pertinent Life History Traits of Smallmouth Bass

Smallmouth bass are predatory fishes, native to the Great Lakes and east central North America (Page and Burr 1991). Each spring, following an increase of water temperature to 12.8°–20.0°C, males move into shallower water and prepare a nest (Scott and Crossman 1973). Ridgway *et al.* (1991) showed that 71.4% of returning males nested within 100 m of nests used in the previous year. Following a ritualized courtship display, females lay approximately 15000 eggs kg⁻¹ of their weight (Scott and Crossman 1973). Wiegmann and Baylis (1995) provide ecological evidence for monogamous mating; however, only males defend the eggs and fry after swim-up. Smallmouth bass mature from 3–6 years of age and can live to 18 years; it is highly probable that generations overlap during spawning. Using DNA fingerprinting techniques, Gross and Kapuscinski (1997) calculated that 5.4% of the spawning males produced 54.7% of the fall young of year. Records indicate that about 40% of nests may fail,

with successful nests yielding up to 2,000 fry (Scott and Crossman 1973). In Lake Erie, nest failure is most often attributable to spring storm events through wave action and water level fluctuations (Trautman 1981), movement of cooler water inshore (Geoff Steinhart, pers. comm.), and predation of eggs and fry by the recently introduced round goby (Steinhart *et al.* 2004a). In addition, larger smallmouth bass males procure more eggs (Wiegmann *et al.* 1992) and therefore produce larger broods (Ridgway and Friesen 1992). Body size also appears important for defending nests from potential predators of eggs and fry (Philipp *et al.* 1997). These factors appear to result in large fitness variances among spawners. After defense of the fry, adult males move into summer home ranges while their age-0 offspring remain within 200 m of the nest (Ridgway *et al.* 2002).

MATERIALS AND METHODS

Sites and Samples

Smallmouth bass were collected at 11 sites that included all three basins of Lake Erie (Fig. 1). Collections were made of spawning (April–June) or young of year populations (September–October, Long Point Bay, ON; LPB). A single collection was made in July (Sandusky Bay, SDY). Smallmouth bass were collected by angling, gill nets, seines, trawls, and electroshocking primarily by state and provincial agencies. Specimens or fin clips were stored either on ice in the field and then at –80°C in the laboratory or placed directly in 95% ethyl alcohol in the field. Genomic DNA was extracted from liver, muscle, or fin clips using a DNeasy[®] tissue extraction kit (Image Inc., Valencia, CA). Sampling sites are abbreviated in the text as follows: Eastern basin: LPB—Long Point Bay, ON; VBB—Van Buren Bay, NY; Central basin: FHR—Fairport Harbor, OH; PRY—Perry, OH; ASH—Ashtabula, OH; CON—Conneaut, OH; Western basin: MBI—Middle Bass Island, OH; SBI—South Bass Island, OH; SDY—Sandusky Bay, OH; PCN—Port Clinton, OH; and GEM—Gem Beach, OH.

The Ontario Ministry of Resources has collected fewer than 30 smallmouth bass in 13 years (1989–2002) along the Ontario coast of Lake Erie in the central basin (Andy Cook, OMNR, pers. comm.). Their scarcity may be due in part to the lack of tributaries entering Lake Erie along the northern shoreline between Point Pelee and Long Point Bay, ON (LPB). Smallmouth bass are inshore fishes, and thus geographical distances among sam-

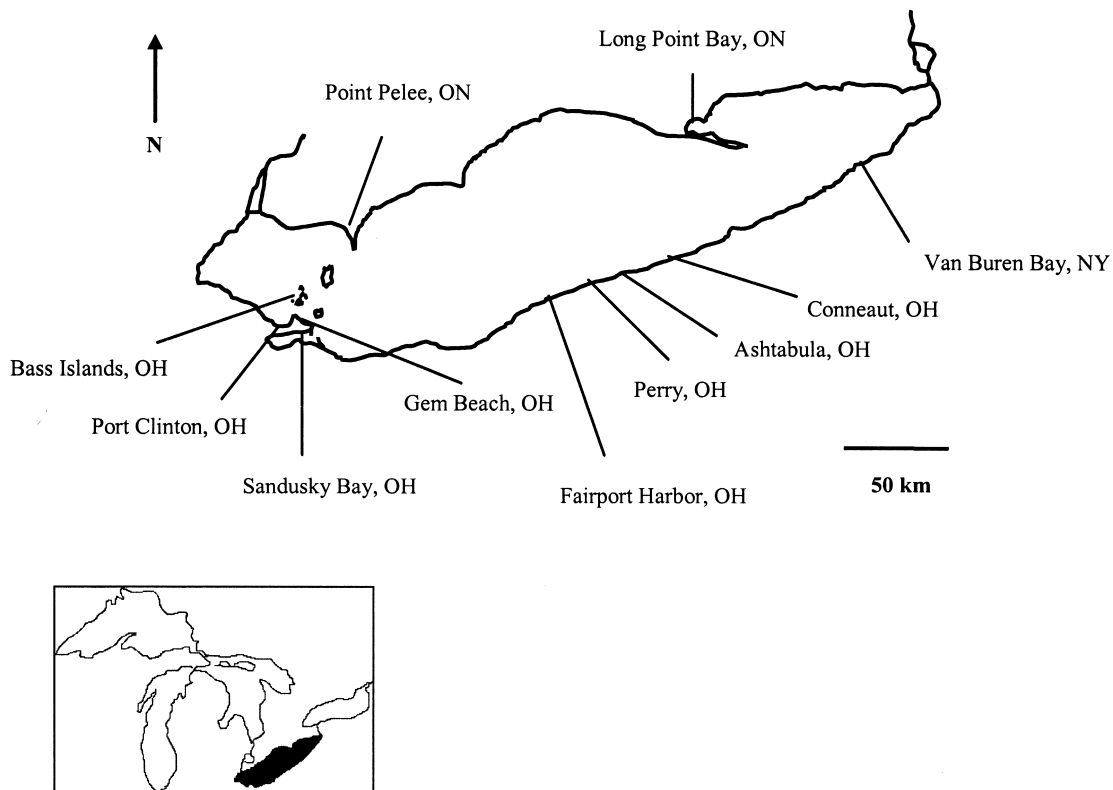


FIG. 1. Sampling site distribution within Lake Erie. Sites of Fairport Harbor, Perry, Ashtabula, and Conneaut are a composite of sites within the immediate area. South Bass Island includes samples from Gibraltar Island; a small island within Put-in-Bay on SBI. Sites are abbreviated in the text as follows: Eastern basin: LPB—Long Point Bay, ON; VBB—Van Buren Bay, NY; Central basin: FHR—Fairport Harbor, OH; PRY—Perry, OH; ASH—Ashtabula, OH; CON—Conneaut, OH; Western basin: MBI—Middle Bass Island, OH; SBI—South Bass Island, OH; SDY—Sandusky Bay, OH; PCN—Port Clinton, OH; GEM—Gem Beach, OH. Inset: Lake Erie (shaded) among the Great Lakes.

pling sites were measured along the shoreline in an arc extending from Point Pelee on the west, along the southern shoreline of Lake Erie, to LPB at the eastern end (Fig. 1). Thus that section of north shore between Point Pelee and LPB was excluded from calculations of pairwise geographical distances, thereby constraining analyses of smallmouth bass migration to the southern shore of Lake Erie.

Mitochondrial DNA

The present study examined sequence variation in the left domain of the control region (~300 bp) and the 3' half of the cytochrome b gene (bp 601–1,140). DNA regions were amplified using polymerase chain reactions (PCR) with a MJ Research PTC-200 thermalcycler in 27 μ L volume re-

actions including 18.5 μ L ddH₂O, 2.7 μ L 10 \times PerkinElmer buffer with MgCl₂ (Novagen Inc., Madison, WI), 2 μ L dNTP mix (0.7 mM), 1 μ L each of forward and reverse primers (0.4 μ M), 0.2 μ L NovaTaq DNA polymerase (Novagen Inc., Madison, WI), and 1–2 μ L genomic DNA. The primer set (forward: 5'-GTGACTTGAAAAAC-CACCGTTG-3', reverse: 5'-CTCCATCTCCGGTT-TACAAGAC-3') designed by Song *et al.* (1998) was used to amplify cytochrome b, and primer sets CB3R-L (5'-CATATTAACCCGAATGATATTT-3') or Pro-L (5'-CTACCTCCAACCTCCCAAAGC-3'; both Palumbi 1996) and HN20-R (5'-GTG CTTATGCTTTAGTTAAGC-3'; Bernatchez and Danzmann 1993) were used for the control region. PCR conditions for mtDNA were 40 seconds denaturation at 94°C, 40 seconds annealing at 52°C (con-

trol region) or 46°C (cyt b), and 90 seconds polymerization at 74°C for 40 cycles. PCR amplicons were visualized using 1.2% agarose gels with ethidium bromide under ultraviolet light, photographed, and subsequently cleaned with either a Qiaquick™ (Qiagen Inc., Valencia, CA) purification kit or ExoSAP-IT® (U.S. Biochemical, Solon, OH).

Automatic sequencing was performed in a single direction using the dideoxy method of termination by Cleveland Genomics, Inc. (using Applied Biosystems, Inc. sequencers) or with a Beckman-Coulter CEQ-8000 capillary analyzer at the Cleveland State University (CSU) DNA Core Facility. Internal primer HW1-R (5'-GTCCTCACCTTCAATAACCG-3'; the complement of HW1-F, Gatt *et al.* 2000) was used for sequencing of the control region. Internal primers basscytb1 (5'-CACCCCTACTTCTCCTACAAAGA-3') and basscytb2 (5'-CCCTCACCCGCTTCTTYGCCTT-3'; the complement of basscytbr1; Near *et al.* 2003) were used for sequencing of cytochrome b. A representative of each haplotype was reamplified and resequenced to minimize possibility of sequencing error. Chromatographs were read by hand and then aligned using Se-Al (Rambaut 1996). No indels were observed and alignment was straightforward.

Microsatellites

Eight microsatellite loci were selected from published literature, of which six (*Mdo2*, *Mdo3*, *Mdo5*, *Mdo8*, *Mdo9*, and *Mdo11*) were chosen from Malloy *et al.* (2000). In order to include loci having greater allelic variation, *RB7* (DeWoody *et al.* 1998) and *MS19* (DeWoody *et al.* 2000) were also employed.

The loci *Mdo2*, *Mdo3*, *Mdo5*, *Mdo8*, *Mdo9*, and *Mdo11* were amplified in a single PCR reaction following the approach to multiplexing by Neff *et al.* (2000), and *RB7* and *MS19* were amplified together following DeWoody *et al.* (2000). Reaction volumes were typically 11 µL for the *RB7/MS19* duplex and 20 µL for the six-plex reaction. Specific aliquot proportions for *RB7/MS19* were 7.4 µL ddH₂O, 1.0 µL 10× PerkinElmer buffer with MgCl₂, 0.75 µL of dNTP mix (0.68 µM), 2 µL each of forward and reverse primers for *RB7* (1.8 µM), 1 µL each of forward and reverse primers of *MS19* (0.9 µM), 0.1 µL NovaTaq DNA polymerase, and 1 µL genomic DNA. The six-plex reaction aliquots included 12 µL ddH₂O, 2.0 µL 10× PE buffer with MgCl₂, 1.2 µL of dNTP mix (0.6 mM), 6 µL each of primer sets for loci *Mdo3* and *Mdo11* (3 µM),

2 µL each of the primer set for *Mdo2* (1 µM), 1.6 µL each of primer sets for loci *Mdo8* and *Mdo9* (0.8 µM), 1.2 µL each of primer sets for *Mdo5* (0.6 µM), 0.2 µL NovaTaq DNA polymerase, and 1.5 µL genomic DNA. Forward primers were dye-labeled for allelic length recognition by the autosequencer.

PCR amplification conditions were an initial denaturation of 94°C for 60 seconds, followed by 40 cycles of 30 seconds denaturation at 94°C, 50 seconds annealing at 47°C (*RB7/MS19*) or 55°C (six-plex), and 30 seconds polymerization at 72°C. PCR products were diluted by 1:20 with sample loading solution and allelic lengths were scored with a Beckman-Coulter CEQ-8000 capillary analyzer with respect to a size standard. Microsatellite allele lengths were also manually checked to the nearest nucleotide. All samples for a given locus were read contemporarily to minimize interpretation errors in allelic lengths. Unresolved loci were repeated; however, some loci could not be resolved with some individuals. A previously analyzed sample was included with each set of samples to check for drift of allele lengths.

Data Analyses

mtDNA variability was described using gene diversity indices and tests for selective neutrality (ARLEQUIN 2.2, Schneider *et al.* 2000). Microsatellites were described by single locus, sampling site, and sampling site by locus diversity indices, tests for linkage disequilibrium among loci, and conformance to Hardy-Weinberg equilibrium expectations within loci [using the exact test of Guo and Thompson (1992)] in GENEPOP (web version 3.4, Raymond and Rousset 1995). Probability (p) values were corrected for multiple tests applied to the same hypothesis using sequential Bonferroni correction (Rice 1989). We followed the approach of Ruzzante *et al.* (1999) and report statistically significant findings both before and after Bonferroni correction. Further, we recognized the arguments of Ward and Grewe (1994) and Waples (1998) that statistical and biological significance are not always congruent when interpreting results.

The hierarchical partitioning of genetic variation according to covariances was examined for the following geographical scheme in Lake Erie: among basins, among sites within basins, and within sites using Analysis of Molecular Variance (AMOVA; Excoffier *et al.* 1992, in ARLEQUIN 2.2). By testing population divergences among samples from

TABLE 1. *Cytochrome b haplotypes by site and basin with haplotype (h) and nucleotide (π) diversities.*

	1	2	3	4	5	Total	h	SE	$\pi \times 100$	SE $\times 100$
Western Basin										
Port Clinton	7	3			1	11	0.56	0.13	0.11	0.11
Gem Beach	6	14	1			21	0.50	0.09	0.10	0.09
Sandusky Bay	7	2				9	0.39	0.16	0.07	0.08
Middle Bass I.		6				6	0.00	0.00	0.00	0.00
South Bass I.		4				4	0.00	0.00	0.00	0.00
						51	0.53	0.04	0.11	0.10
Central Basin										
Fairport Harbor	7	1				8	0.43	0.17	0.08	0.09
Perry	4	7				11	0.51	0.10	0.09	0.10
Ashtabula	7					7	0.00	0.00	0.00	0.00
Conneaut	6	2				8	0.43	0.17	0.08	0.09
						34	0.43	0.07	0.08	0.08
Eastern Basin										
Van Buren Bay, NY	8	6		1		15	0.59	0.08	0.12	0.11
Long Point Bay, ON	11	2				13	0.28	0.14	0.05	0.07
						28	0.47	0.08	0.09	0.09
haplotype total	63	47	1	1	1	113				
haplotype frequency	0.56	0.42	0.01	0.01	0.01		0.52	0.02	0.10	0.09

the eastern, central, and western basins, we examined the hypothesis that bathymetric structure in Lake Erie has influenced population structure of smallmouth bass.

Goodman (1997) proposed that if evaluating population differentiation is the research goal, then an allelic goodness of fit test is appropriate. To this end, population differentiation tests based on allele distributions of mtDNA sequences (ARLEQUIN 2.2) and microsatellite loci (GENEPOP, web version 3.4) were performed following the methods of Raymond and Rousset (1995). Population divergence was quantified using estimates of F_{ST} (Wright 1969) in order to calculate the number of migrants and to analyze the distribution of genetic diversity relative to geographic distribution (Goodman 1997). Population divergence based on mtDNA was estimated using Φ_{ST} (Weir and Cockerham 1984; a measure analogous to F_{ST} as demonstrated by Hudson *et al.* 1992) and from microsatellites using ρ_{ST} (allele size; Rousset 1996) and θ_{ST} (allele identity; Weir and Cockerham 1984). Negative divergence estimates were an artifact of calculation and therefore were converted to "zero." Comparison of θ_{ST} and ρ_{ST} estimates can reveal the relative importance of genetic drift versus mutation. If θ_{ST} and ρ_{ST} estimates are similar, then genetic drift has likely played a more promi-

nent role, whereas stepwise mutations outweigh drift if ρ_{ST} estimates are greater than θ_{ST} estimates (Hardy *et al.* 2003). To test for the effect of several small sample sizes particularly in the microsatellite data set, an additional analysis with a reduced number of sites (7, by pooling adjacent sites) was conducted. Pairwise microsatellite and mtDNA population divergence estimates were compared statistically using a Wilcoxon matched pairs test in STATISTICA v.6 (StatSoft 2001). Number of migrants was calculated from quantified estimates of population divergence using $N_{fm} = [((1/F_{ST}) - 1)/2]$ and $N_m = [((1/F_{ST}) - 1)/4]$ for mtDNA and microsatellites respectively. Both derivations assumed an island model of migration (Wright 1951), population equilibrium, and selective neutrality.

Neighbor-joining trees (Saitou and Nei 1987) were constructed with PAUP*4b10 (Swofford 1998) using Nei's corrected average pairwise differences (d_A , 1987, equation 10.21) in ARLEQUIN 2.2. Minimum spanning trees depicting overall similarity among sites (MST, Kruskal 1956) were drawn by hand from estimates of population divergence. Isolation by distance among sampling sites was evaluated under a model of stepping stone migration (Kimura and Weiss 1964) by regressing Slatkin's linearized F_{ST} estimates ($F_{ST}/(1 - F_{ST})$) against pairwise logarithmic geographic distances

TABLE 2. Control region haplotypes by site and basin with haplotype (*h*) and nucleotide (π) diversities.

	1	2	3	4	5	6	7	8	Total	<i>h</i>	SE	$\pi \times 100$	SE $\times 100$
Western Basin													
Port Clinton	11	3	2	2					18	0.61	0.11	0.36	0.28
Gem Beach	12	1							13	0.15	0.13	0.10	0.13
Sandusky Bay	6	5	2						13	0.67	0.08	0.49	0.36
Middle Bass I.	4			1	1				6	0.60	0.22	0.29	0.27
South Bass I.	3			2					5	0.60	0.18	0.20	0.22
									55	0.54	0.07	0.32	0.25
Central Basin													
Fairport Harbor	7	1			1		1		10	0.53	0.18	0.33	0.27
Perry	5	2		1					8	0.61	0.16	0.35	0.29
Ashtabula	2	2		1					5	0.80	0.16	0.47	0.40
Conneaut	3		3	1			1		8	0.79	0.11	0.45	0.35
									31	0.67	0.08	0.40	0.29
Eastern Basin													
Van Buren Bay, NY	8	1		2			1		12	0.56	0.15	0.26	0.23
Long Point Bay, ON	7	1	1	2		2		1	14	0.75	0.11	0.41	0.31
									26	0.65	0.10	0.34	0.26
haplotype total	68	16	8	12	2	2	3	1	112				
haplotype frequency	0.61	0.14	0.07	0.11	0.02	0.02	0.03	0.01		0.60	0.05	0.35	0.26

(Rousset 1997) using the ISOLDE program (GENEPOP 3.4) and 10,000 permutations of the Mantel test. A non-significant relationship suggests genetic drift and not gene flow has played a more prominent role in population divergence (Hutchinson and Templeton 1999).

RESULTS

mtDNA Sequences

Five mtDNA cytochrome *b* gene haplotypes (Genbank Accession numbers DQ354383-DQ354387) from 113 individuals were defined by four polymorphic sites; all were synonymous changes in the third codon position. Two haplotypes characterized 98% of the smallmouth bass sampled in Lake Erie and occurred in almost every sampling site (Table 1). Eight mtDNA control region haplotypes (Genbank Accession numbers DQ354375-DQ354382) from 112 individuals were defined by six polymorphic sites. A single control region haplotype was found at all sampling sites (Table 2), characterizing 61% of smallmouth bass sampled from Lake Erie. Selective neutrality (Tajima's *D*: Tajima 1989; Ewens-Watterson: Ewens 1972, Watterson 1975) was supported for both the control region and cytochrome *b* gene, suggesting that observed heterozygosity levels were explainable by

a nearly neutral model of evolution. Greater genetic diversity was found in the western basin samples using the cytochrome *b* gene (Table 1), whereas haplotype and nucleotide diversities were greatest in the central basin of Lake Erie in the control region data (Table 2). Neither trend was significant.

Partitioning of genetic variance among three hierarchical levels revealed that the greatest covariation occurred within sampling sites: 97.9% ($p = 0.14$, $df = 112, 11$) and 73.2% ($p < 0.00001$, $df = 113, 11$) for the control region and the cytochrome *b* gene, respectively. Measures of covariation among sampling sites within lake basins were 4.6% ($p = 0.10$, $df = 11, 3$) and 24.9% ($p < 0.00001$, $df = 11, 3$), whereas those among the three basins were negligible (0%, $p = 0.92$; 1.9%, $p = 0.29$; $df = 3$) for the control region and cytochrome *b* data. At the lake basin level, the western basin was significantly divergent from the central basin ($\Phi_{ST} = 0.13$, $p = 0.01$) and the eastern basin ($\Phi_{ST} = 0.12$, $p = 0.01$) in the case of the cytochrome *b* gene data only. Tests of population differentiation were consistent with divergence estimates identifying the western basin as divergent from both the central and eastern basins.

Divergences between pairs of populations based on the control region data identified three nominally divergent pairs of sites ($p < 0.05$; GEM—

TABLE 3. Divergent sampling sites based on tests of population differentiation partitioned into location by basin. Underlined pairs significant after Bonferroni correction (initial $\alpha = 0.0009$). Superscript number is microsatellite locus identification. Loci *Mdo3* and *Mdo5* identified no statistically significant pairs. *Mdo9*, *Mdo11*, and *MS19* were identified as statistically significant loci. “+” indicates nominal significance ($p < 0.05$), and “*” indicates statistical significance following Bonferroni correction pooled over all loci. Number in parentheses is the number of site-site comparisons, and multiplied by eight for microsatellites.

Western basin (10)	Central basin (6)	Eastern basin (1)	W-C (20)	W-E (10)	C-E (8)
<i>Control region</i>					
GEM-SDY			GEM-ASH GEM-CON		
<i>Cytochrome b</i>					
MBI-PCN	PRY-ASH		GEM-ASH	GEM-LPB	
SDY-MBI			<u>MBI-ASH</u>	MBI-LPB	
SDY-SBI			MBI-CON	MBI-VBB	
			MBI-FHR	PRY-LPB	
			SBI-ASH	SBI-LPB	
<i>Microsatellites</i>					
SDY-MBI ^{RB7}	CON-ASH ²	<u>LPB-VBB</u> ^{9*}	PCN-CON ²	PCN-LPB ^{RB7}	CON-LPB ⁸⁺
SDY-MBI ⁹	CON-FHR ²	<u>LPB-VBB</u> ¹¹	GEM-CON ⁸	GEM-VBB ^{RB7+}	<u>ASH-LPB</u> ⁹⁺
SDY-GEM ¹¹	CON-PRY ²	<u>LPB-VBB</u> ^{MS19}	MBI-ASH ⁹	<u>GEM-LPB</u> ^{9*}	CON-LPB ⁹
SDY-MBI ¹¹	PRY-ASH ⁹		MBI-ASH ¹¹	MBI-VBB ⁹	<u>FHR-LPB</u> ^{9*}
SDY-PCN ¹¹⁺	PRY-FHR ⁹		PCN-ASH ¹¹	<u>PCN-LPB</u> ^{9*}	PRY-LPB ⁹⁺
			PCN-CON ¹¹	<u>SBI-LPB</u> ⁹⁺	ASH-VBB ⁹
			PCN-PRY ¹¹	<u>SDY-LPB</u> ^{9*}	ASH-LPB ¹¹
				GEM-VBB ¹¹	PRY-LPB ¹¹
				MBI-VBB ¹¹	<u>FHR-LPB</u> ^{MS19}
				PCN-VBB ¹¹⁺	PRY-LPB ^{MS19}
				<u>SDY-LPB</u> ¹¹	
				<u>SDY-VBB</u> ¹¹	
				<u>GEM-LPB</u> ^{MS19}	
				MBI-LPB ^{MS19}	
				<u>PCN-LPB</u> ^{MS19}	
				<u>SDY-LPB</u> ^{MS19}	
				<u>SDY-VBB</u> ^{MS19}	

ASH, CON, SDY), but probabilities were not significant after Bonferroni correction. Likewise, population differentiation tests using the cytochrome b data identified 14 nominally significant site comparisons (Table 3; $p < 0.05$, of 55 possible comparisons), but only a single pair was significant after Bonferroni correction (MBI—ASH, $p = 0.0008$). Overall, populations were weakly divergent as evidenced by an average $\Phi_{ST} = 0.05$ for the control region data, but more divergent (average $\Phi_{ST} = 0.27$) using cytochrome b data. Pairwise Φ_{ST} values for the control region ranged from 0.00 to 0.31 and from 0.00 to 1.00 for the cytochrome b gene. Wilcoxon matched pairs tests indicated that Φ_{ST} estimates derived independently from the control re-

gion and the cytochrome b gene were significantly different ($Z = 4.06$, $p < 0.0001$). Given the disparate results from the control region and the cytochrome b gene, the data sets were not combined into a single haplotype for analysis.

Microsatellites

Numbers of alleles per locus ranged from three (*Mdo3*, *Mdo5*, *Mdo11*) to 10 (*MS19*) and observed heterozygosities (H_O) from 0.11 to 0.66 (Table 4). Locus *Mdo11* pooled over sampling sites ($p = 0.03$) and two sampling sites pooled over loci (LPB, $p = 0.01$; GEM, $p = 0.03$) were not within Hardy-Weinberg expectations prior to Bonferroni correction, but corresponded following p-value correction.

TABLE 4. Summary statistics for 11 sampling sites of smallmouth bass, *Micropterus dolomieu*, in Lake Erie; including sample size (n), number of alleles (N_A) per locus and site, mean allele size in nucleotides, observed and expected heterozygosities (H_O and H_E), and estimate of F_{IS} from Weir and Cockerham (1984; negative values = heterozygote excess, positive values = heterozygote deficiency). A significant departure from Hardy-Weinberg equilibrium after Bonferroni correction at sampling site GEM, locus Mdo9 is denoted by “*”.

		PCN	GEM	SDY	MBI	SBI	FHR	PRY	ASH	CON	VBB	LPB	
Locus													
Mdo2	212	n	27	25	20	8	8	15	26	4	9	36	34
	4	N _A	2	2	3	2	2	2	2	1	2	2	4
	197.9	allele size	197.7	198.0	198.3	198.0	197.8	197.7	197.8	197.0	199.0	198.0	197.9
	0.363	H _O	0.370	0.480	0.450	0.500	0.125	0.333	0.231	—	0.556	0.333	0.382
	0.373	H _E	0.307	0.372	0.465	0.400	0.325	0.287	0.317	—	0.529	0.380	0.431
	0.027	F _{IS}	-0.209	-0.297	0.034	-0.273	0.632	-0.167	0.275	—	-0.053	0.125	0.114
Mdo3	189	n	22	29	16	9	8	5	22	3	4	49	22
	3	N _A	2	3	3	3	2	2	3	2	2	3	3
	118.8	allele size	118.5	119.0	119.2	119.2	118.8	118.2	118.9	118.7	119.0	118.8	118.8
	0.413	H _O	0.182	0.448	0.562	0.778	0.250	0.200	0.500	0.667	0.000	0.428	0.364
	0.474	H _E	0.406	0.520	0.558	0.582	0.500	0.200	0.487	0.533	0.571	0.455	0.449
	0.129	F _{IS}	0.558	0.140	-0.008	-0.366	0.517	0.000	-0.027	-0.333	1.000	0.058	0.194
Mdo5	232	N	25	20	17	8	8	31	24	7	11	46	35
	3	N _A	2	3	3	2	2	2	2	1	2	2	2
	198.9	allele size	198.9	198.8	198.7	198.9	198.9	198.9	199.0	198.7	199.0	198.8	198.9
	0.108	H _O	0.080	0.150	0.235	0.125	0.125	0.032	0.042	0.286	—	0.174	0.057
	0.126	H _E	0.078	0.145	0.219	0.125	0.125	0.094	0.042	0.264	—	0.196	0.109
	0.142	F _{IS}	-0.021	-0.036	-0.076	0.000	0.000	0.659	0.000	-0.091	—	0.113	0.481
Mdo8	195	n	27	19	15	8	6	12	26	4	8	33	37
	8	N _A	6	6	7	5	3	5	5	4	6	6	6
	216.2	allele size	215.7	216.4	216.8	215.9	216.3	216.5	216.5	214.3	215.3	216.7	215.9
	0.662	H _O	0.667	0.842	0.600	0.625	0.167	0.667	0.577	0.750	0.750	0.727	0.649
	0.710	H _E	0.732	0.688	0.696	0.792	0.621	0.696	0.678	0.896	0.817	0.758	0.667
	0.069	F _{IS}	0.091	-0.231	0.143	0.222	0.75	0.043	0.152	0.1	0.087	0.042	0.028
Mdo9	241	n	26	28	18	10	11	14	30	7	11	45	41
	5	N _A	4	3	2	2	3	3	2	3	2	3	3
	124.8	allele size	128.6	128.3	127.8	129.2	127.9	128.2	128.6	128.9	128.5	128.0	129.5
	0.473	H _O	0.654	0.428 *	0.556	0.200	0.545	0.714	0.567	0.571	0.545	0.467	0.220
	0.506	H _E	0.533	0.564	0.508	0.337	0.567	0.606	0.463	0.659	0.485	0.538	0.240
	0.065	F _{IS}	-0.232	0.243	-0.097	0.419	0.040	-0.187	-0.229	0.143	-0.132	0.133	0.089
Mdo11	192	n	24	26	19	6	4	19	23	7	8	47	19
	3	N _A	2	3	2	2	2	3	3	2	3	3	3
	173.4	allele size	172.8	173.0	173.9	172.3	173.5	173.7	173.7	174.0	173.5	173.8	172.5
	0.411	H _O	0.333	0.308	0.316	0.167	0.250	0.444	0.565	0.143	0.250	0.660	0.211
	0.503	H _E	0.337	0.391	0.512	0.167	0.536	0.569	0.559	0.538	0.608	0.606	0.284
	0.183	F _{IS}	0.011	0.217	0.390	0.000	0.571	0.229	-0.011	0.750	0.606	-0.089	0.265
RB7	248	n	31	28	20	7	12	19	28	7	13	40	43
	7	N _A	6	4	5	4	3	5	4	3	5	6	5
	129.8	allele size	128.7	129.0	130.0	129.9	130.3	130.5	129.5	131.3	128.2	128.5	126.5
	0.629	H _O	0.613	0.536	0.600	0.571	0.750	0.684	0.678	0.571	0.769	0.575	0.651
	0.642	H _E	0.660	0.691	0.562	0.703	0.638	0.630	0.650	0.648	0.674	0.612	0.621
	0.020	F _{IS}	0.072	0.228	-0.070	0.200	-0.186	-0.088	-0.045	0.127	-0.148	0.061	-0.049
MS19	263	n	31	29	19	9	13	28	27	7	13	42	45
	10	N _A	7	4	5	3	3	5	6	4	3	5	4
	103.0	allele size	102.9	103.6	103.8	102.4	102.8	103.3	103.0	103.1	102.2	103.6	102.3
	0.555	H _O	0.516	0.483	0.789	0.556	0.462	0.536	0.556	0.714	0.615	0.667	0.422
	0.557	H _E	0.584	0.585	0.691	0.569	0.520	0.567	0.558	0.571	0.465	0.603	0.375
	0.003	F _{IS}	0.118	0.177	-0.146	0.024	0.117	0.056	0.004	-0.277	-0.343	-0.108	-0.127

Only GEM at *Mdo9* did not conform to HW expectations following Bonferroni correction (Table 4), which may be due to chance. Conformance to HW expectations suggested temporal heterogeneity across collections years within a sampling site may not be a confounding factor. All locus-locus pairwise comparisons for each population were in linkage equilibrium. Private alleles were identified for both individual sampling sites and within basins: *Mdo2* (LPB), *Mdo5* (western basin), *Mdo8* (GEM), *Mdo9* (SBI), two alleles at *RB7* (PCN, eastern basin), and three at *MS19* (one allele at FHR, and two alleles at PCN). In addition, an *Mdo2* allele was found only in the eastern and western basins, single alleles of both *Mdo9* and *MS19* were shared between the central and western basins, and one allele of *MS19* appeared only in the eastern and central basins. Each private allele had a frequency less than 5% either within its respective sampling site, basin, or shared between basins. Allele frequencies are available from the authors.

AMOVA analysis showed that all covariation occurred within sampling sites for θ_{ST} and ρ_{ST} (100%, $p = 1.0$, $df = 603, 8$). ρ_{ST} estimates failed to identify any divergence among basins although population differentiation tests identified statistical divergence between the western and central basins ($p = 0.04$) prior to Bonferroni correction but not following correction ($\alpha = 0.02$). Global θ_{ST} and ρ_{ST} estimates were not significantly different ($n = 55$, $Z = 0.30$, $p = 0.76$). Pairwise population divergence estimates averaged 0.01 and ranged from 0.00 to 0.04 (θ_{ST}) or 0.07 (ρ_{ST}). Despite these relatively low divergence levels, loci *Mdo9*, *Mdo11*, and *MS19* indicated differentiation among sampling sites. Pairwise site comparisons identified 28 nominally significant pairs (Table 3; of 440 site-pairs comparisons, 55 comparisons by 8 loci) and 16 pairs (nine unique pairs) as statistically significant following Bonferroni correction. Of interest, the two eastern basin sites were divergent from each other and one of these sites, LPB, was involved in 14 of the 16 significant comparisons (Table 3). Population differentiation tests pooled over all loci identified 12 nominally significant site pairs, five of which remained statistically significant following Bonferroni correction (Table 3).

An additional analysis was performed to assess the potential impact of small sample sizes in estimating population divergence. Several sites (Middle and South Bass Island, Port Clinton and Gem Beach, Fairport Harbor and Perry, Conneaut and Ashtabula) were pooled based on geographical

proximity and results of the first analysis. Population differentiation tests of the control region data identified a single, nominally divergent site pair (SDY—Bass Islands). Population differentiation tests of the cytochrome b data identified eight, nominally divergent site pairs (Bass Islands with all other sites plus LPB—ASH/CON, GEM/PCN). Three site pairs remained statistically significant after Bonferroni correction: Bass Islands—ASH/CON, LPB, and SDY. Microsatellite data detected nine, nominally significant site pairs (SDY—GEM/PCN, FHR/PRY; LPB with all six sites; VBB—GEM/PCN). Six site pairs remained significant following Bonferroni correction (exceptions being LPB—Bass Islands; SDY—GEM/PCN; FHR/PRY). AMOVA analyses of all three data sets detected significant within-site and among-site-within-basin covariance partitions in only the cytochrome b data. The largest covariation occurred within-sites, and in microsatellites, all covariation occurred within-sites.

Comparison of mtDNA and Microsatellites

Population divergence estimates derived from mtDNA and microsatellites were statistically different (Φ_{ST} -control vs. θ_{ST} : $Z = 3.64$, $p < 0.001$; Φ_{ST} -control vs. ρ_{ST} : $Z = 4.06$, $p < 0.0001$; Φ_{ST} -cyt b vs. θ_{ST} : $Z = 5.58$, $p < 0.000001$; Φ_{ST} -cyt b vs. ρ_{ST} : $Z = 5.73$, $p < 0.000001$). Average Φ_{ST} was 0.27 and 0.05 for cytochrome b and control region, respectively. Divergence estimates using cytochrome b data were $\sim 5\times$ greater than those from the control region and $\sim 25\times$ greater than those from microsatellites. Those based on the control region were $\sim 5\times$ those of microsatellites. Cytochrome b data yielded larger estimates of population divergence than did the more variable control region and microsatellite loci, although microsatellites revealed a greater number of significantly divergent sampling site-pairs. Genetic relationships among sampling sites differed based on the molecular marker with respect to the number of statistically significant or identification of site-pairs.

Geography of Genetic Variation

Neighbor-joining trees depicting relationships among sampling sites showed little topological similarity among the data sets (Fig. 2). Trees were reflective of the small genetic distances among sites including the presence of negative branch lengths. As a consequence, topological solutions were not

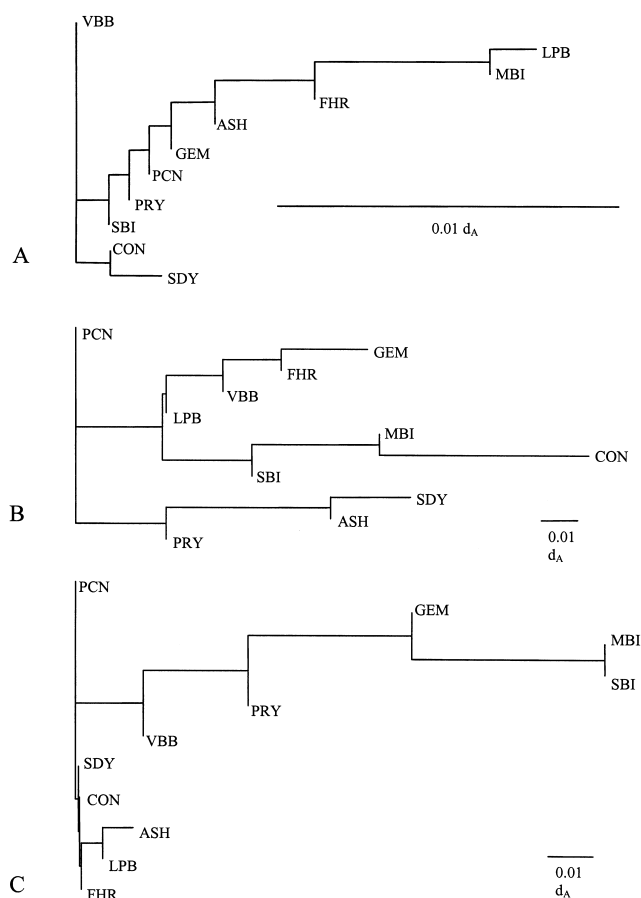


FIG. 2. Neighbor-joining trees derived from d_A (Nei's 1987 corrected average pairwise difference) for (A) pooled microsatellite loci, (B) control region sequences, and (C) cytochrome b sequences. Sampling site abbreviations follow notation in Figure 1. Topologies do not represent unique solutions. Negative genetic distances (branch lengths) converted to "zero."

unique. As an alternative, a minimum spanning tree among sampling sites was constructed by hand using F_{ST} estimates. MST's derived from control region sequences and microsatellites consisted of a single genetic entity (trees not shown). However, cytochrome b sequences identified four genetically divergent clusters of sampling sites: Bass Islands, Ashtabula, Gem Beach with Perry, and all other sampling sites (Fig. 3). Geographically adjacent sites on the Bass Islands comprised a single cluster (MBI/SBI), whereas eastern basin sites were grouped with both the central and the remaining western basin sites. Visual inspection of cytochrome b haplotype frequencies supported signif-

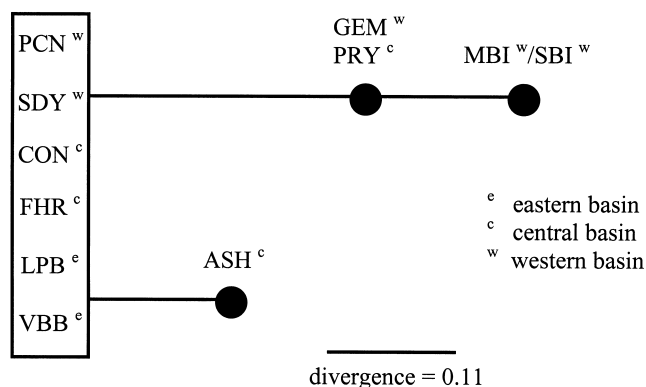


FIG. 3. Minimum spanning tree among sampling sites constructed from Φ_{ST} values of cytochrome b. All sites within the same box are genetically similar, and negative divergence estimates were converted to zero. Distance between boxes is the average Φ_{ST} value for all pairwise distances between sites within two boxes. MST's derived from control region sequences and microsatellites were unresolved.

icantly non-zero genetic distances at the basin and sampling site (Table 1) levels.

Isolation by distance tests detected significant relationships between population divergence ($F_{ST}/(1-F_{ST})$) and $\ln(\text{geographic distance})$ in microsatellite data using both ρ_{ST} ($r^2 = 0.02$, $p = 0.04$) and θ_{ST} ($r^2 = 0.02$, $p = 0.03$) but not using mtDNA sequences.

DISCUSSION

Patterns of Genetic Variation

Although indices of genetic variation and population divergence derived from mtDNA and microsatellites were low, they appear consistent with other aquatic fauna inhabiting previously glaciated areas relative to unglaciated areas (Bernatchez and Wilson 1998). The mean number of microsatellite alleles per locus (5.4) and heterozygosity per locus (0.45) in smallmouth bass fell within the ranges of 13 species of freshwater fishes (9.1 ± 6.1 and 0.54 ± 0.25 respectively, DeWoody and Avise 2000). Heterozygote deficiency at locus *Mdo11* in Lake Erie smallmouth bass is consistent with both smallmouth and spotted (*M. punctulatus*) bass heterozygote deficiencies at the same locus from Broken Bow reservoir in Oklahoma (Malloy *et al.* 2000). Additionally, microsatellite locus heterozygosities reported here for Lake Erie are comparable to locus heterozygosities with similar allele numbers in

yellow perch from Lake Michigan (Miller 2003). Average divergence values derived from microsatellites (θ_{ST} , $\rho_{ST} = 0.01$) and the control region ($\Phi_{ST} = 0.05$) were markedly less than those from the cytochrome b gene, which may be explained by higher mutation rates, as occur in microsatellites relative to mtDNA, and thus lower F_{ST} estimates (Rousset 1996), as well as differences in their relative effective population sizes (summarized in Avise 2004). Within mtDNA, different regions display different evolutionary patterns due to different substitution rates and mutation constraints (Graur and Li 2000).

Sample sizes for microsatellite loci are lower than Ruzzante's (1997) suggestion of 50 to 100 samples per site. Malloy *et al.* (2000) tested six of the microsatellite loci used in this study (*Mdo* series) on smallmouth bass from a reservoir in Oklahoma and found either the same number of alleles per locus (*Mdo8*, *Mdo9*, *Mdo11*), or one fewer allele (*Mdo5*). The two other loci (*Mdo2*, *Mdo3*) had fewer alleles in Lake Erie populations compared to Oklahoma populations (Malloy *et al.* 2000, $N_A = 6$ in both loci). For these loci, the Malloy *et al.* (2000) study was based on sample sizes up to 157, while the present study contains no less than 190 samples per locus. Fish populations in formerly glaciated areas tend to have lower genetic diversity than found in southerly-unglaciated regions (Bernatchez and Wilson 1998). The smallmouth bass populations from Oklahoma in the Interior Highlands contain most of the genetic diversity of the species based on allozyme data (Stark and Echelle 1998). Given the comparable numbers of alleles per locus between smallmouth bass from Lake Erie (formerly glaciated population) and Oklahoma (Interior Highland population), we conclude that most of the genetic variation is being detected with the current sample sizes in Lake Erie in at least four of the six loci. Additional sampling may be necessary to reveal subtle divergences that reflect the migratory patterns of spawning males and summer adults, a question that merits further study.

On a larger geographical scale, the possibility of multiple genetic origins of smallmouth bass in Lake Erie was revealed by analyses of control region and cytochrome b haplotypes. Dhondt (1996) recognized that mtDNA is less variable than microsatellites and is therefore better suited to clarify relationships among populations over larger geographical distances. Furthermore, protein coding genes maintain a higher signal to noise ratio for older geological and biological events that may ex-

plain greater divergences in cytochrome b relative to the control region and microsatellites.

Multiple genetic sources within the Great Lakes and adjacent waterways likely reflect historical events of founding from one or more glacial refugia. This interpretation appears consistent with observed genetic diversity found in lake trout (*Salvelinus namaycush*, Wilson and Hebert 1996), brown bullhead (*Ameiurus nebulosus*, Murdoch and Hebert 1997, and additional references therein), walleye (*Sander vitreus*, Stepien and Faber 1998), and yellow perch in North America (Ford and Stepien 2004). Following postglacial recolonization of the Great Lakes, migration and dispersal patterns, reproductive behavior, and genetic drift likely modified and shaped genetic patterns. Historical effects on mtDNA variability in smallmouth bass revealed at least two genetic groups of mtDNA haplotypes in our data, which may represent the prefragmentation relationships of populations (Johnson *et al.* 2003).

Patterns of Genetic Structure

From the lack of consistent, significant divergences at the basin level across three data sets, bathymetric features partitioning Lake Erie into three basins appear to contribute minimally to the population structure of smallmouth bass. This is consistent with their inshore habitat and geographical dispersal in a linear array (significant isolation by distance model in microsatellites), suggesting that the coastline and the location of tributaries are more influential in smallmouth bass distribution. The latter has been implicated in explaining the paucity of smallmouth bass on the north shore of the central basin. Walleye display some correspondence to basin structure in Lake Erie, with additional divergence occurring among some individual spawning sites in lake reefs and associated tributaries based upon mtDNA sequences (Stepien and Faber 1998, Stepien *et al.* 2004) and microsatellite data (Strange and Stepien, in review).

Some fine-scale divergence among sites was discerned, but sites appeared only weakly divergent with present sample sizes. Genetic drift may have played a more prominent role in population divergence than either stepwise mutation in microsatellite data (n.s. global $\theta_{ST} - \rho_{ST}$ comparison) or gene flow in mtDNA data (n.s. relationship between site-pair divergences and distances). Mitochondrial DNA and nDNA data sets were not consistent in their overall pattern of population structure, but dis-

similarities could have been exacerbated by the possibility of multiple genetic origins. Despite their lack of specific concordance, several sites were consistently identified as genetically distinct: Ashtabula, Bass Islands, Long Point Bay, and Sandusky Bay. It is noteworthy that while the Bass Islands and Sandusky Bay are in close geographical proximity within the western basin, they appear to be genetically divergent. The western basin is characterized by shallow water (< 30 m) and contains many rocky shoals, islands, and reefs suitable as spawning sites. The combination of the relatively large area of spawning habitats in the western basin and the possibility for nest site fidelity may have contributed to divergence between geographically proximate populations. A similar association of little population divergence (R_{ST} : 0–0.1), but significant divergence between bay and lake spawning groups was detected among yellow perch in Lake Michigan suggesting that unique circulation patterns in bays have contributed to population isolation (Miller 2003).

Ward and Grewe (1994) and Waples (1998) point out that failure to reject the null hypothesis of no significant population structure does not imply a single panmictic stock, and Carvalho and Hauser (1994) provide several reasons why stock structure may not be detected. A conservative management approach would be based on the greatest population structure discerned from each data source. In the case of smallmouth bass in Lake Erie, genetic differences across basins and among geographically adjacent sites in spawning groups are present, albeit for different reasons.

Migration and Site Fidelity

Male site fidelity of smallmouth bass has been demonstrated in the field, but philopatry has yet to be resolved empirically. Gross *et al.* (1994), however, inferred philopatry to explain patterns of genetic variation within and among nests in Lake Opeongo. Population genetic theory suggests that a high degree of homing fidelity could result in substantial numbers of private alleles and/or a few shared alleles given sufficient evolutionary time. This pattern was not observed during the present study. Confounding factors include the relatively short postglacial history of smallmouth bass in Lake Erie (~ 14,000 years, or ~ 4,500 generations assuming a 3-year maturation) and thus potentially insufficient time for population divergence and mutation-drift equilibrium to have occurred or the rela-

tively small sample sizes for microsatellite loci. Although private microsatellite alleles were present, no discernable geographic pattern was apparent and all private alleles occurred at frequencies of less than 5%. Possible differences between the sexes in site fidelity may obfuscate patterns and should be tested.

Tagging and recapture studies by the Ohio Division of Wildlife (ODW 2004) revealed minimal movement of individuals during April–June of 1988–2002 (Fig. 4); as well as differential distribution of males and females during June 2003, when 226 (95%) of 237 smallmouth bass caught were females. The ODW (2004) postulated that male smallmouth bass movement was limited due to nesting behavior, and females were found offshore before moving onto nests defended by males. Overall, the behaviors of male nest-site fidelity and adult summer ranges were not revealed in the genetic data. Only a significant isolation by distance pattern detected in the microsatellite data fits the assumption that migration occurs along the shoreline of Lake Erie and supports the field data that long distance migration is rare. Given the apparent lack of migration barriers in the lake, restricted gene flow leading to isolation by distance agrees with the behavior of male nest site fidelity. Under a modified stepping stone model proposed by Gold *et al.* (2001), geographically adjacent populations share greater gene flow and thus lack tightly circumscribed boundaries, instead showing more regional clustering patterns.

Extrapolation of divergence estimates to calculate the number of migrants was considered tenuous given the disparity in the divergence estimates among data sets, with N_m ranging from 0 to “infinity” for both mtDNA and microsatellites. Despite large migrant estimates, some sites within the same basin were genetically divergent, which is in agreement with Wright’s (1969) prediction that population divergence is possible despite migration. Up to 50 individual migrants per generation yielded statistical divergence in 50% of simulations by Allendorf and Phelps (1981). However, the potential for large variances in the reproductive success of smallmouth bass may confound our conclusions by artificially inflating divergence values and thus underestimating migration rates, especially when sampling from juvenile populations (Allendorf and Phelps 1981, Waples 1998): the “Allendorf-Phelps” effect.

This A.-P. phenomenon may have applicability in samples analyzed from Long Point Bay, ON. This

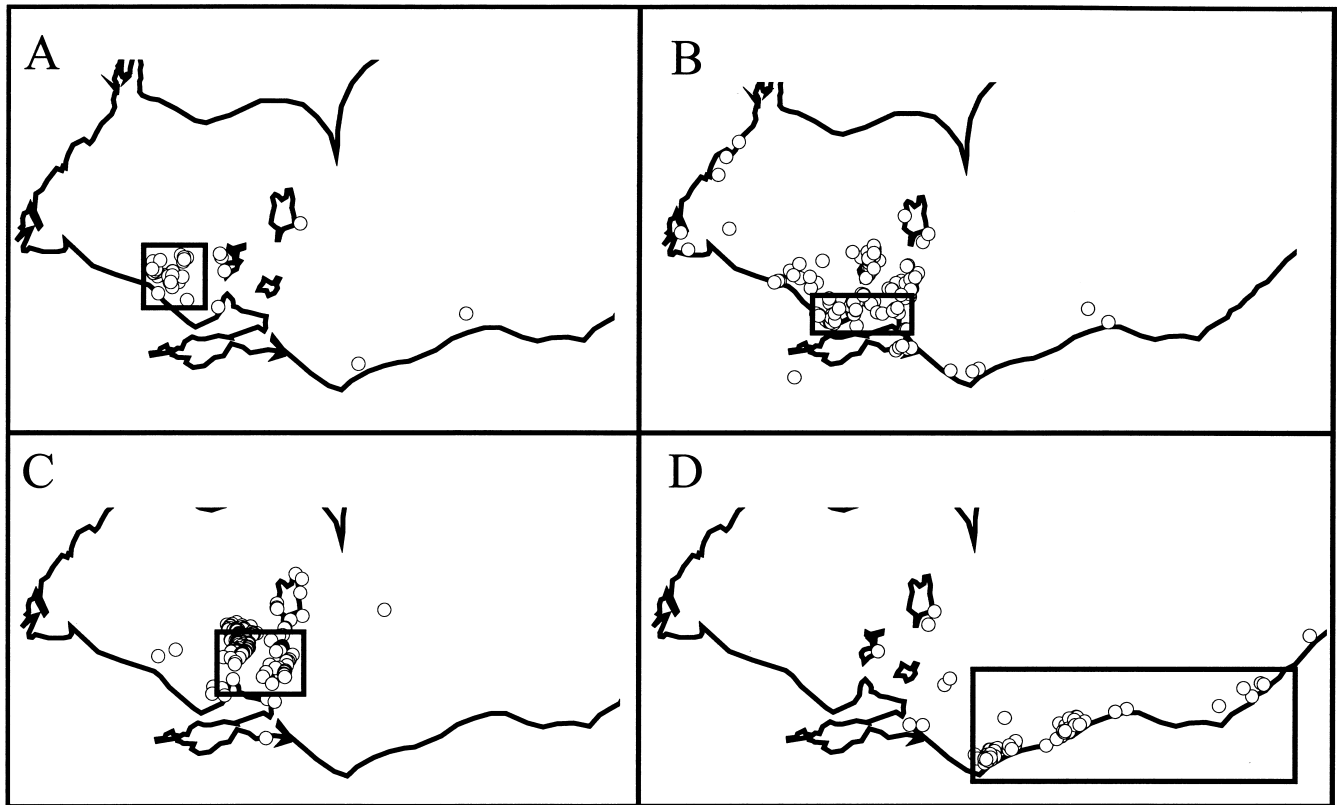


FIG. 4. Smallmouth bass tagging areas (boxed) and recapture locations (open circles) for 1998–2002. Tagging areas are (A) western basin reef complex, (B) nearshore areas from Port Clinton to Lakeside, OH, (C) Bass Islands and Kelley's Island, and (D) central basin from Huron to Cleveland, OH. (Reproduced with permission from Kevin Kayle, Fairport Harbor Fisheries Research Unit, ODW, ODNR).

sample consisted of young of year fish (YOY or age-0). It was not in HWE (before Bonferroni correction) and was involved in 14 of the 16 pairwise comparisons identified by microsatellites statistically differing from other sampling sites. If the “Allendorf-Phelps” effect has artificially inflated divergence estimates at LPB, then most of the detected divergence that we found here is not representative of true population differences. Removal of site comparisons involving LPB, as it is a safe assumption that the YOY collection represents fish spawned within 200 m of their nest of origin (Ridgway *et al.* 2002), reduced population divergence in microsatellites to levels comparable with mtDNA.

CONCLUSIONS

Although tagging and recapture data suggest that behaviorally defined spawning stocks may exist, the present study suggests only slight population genetic divergences and few pairwise differences

among sites. Additional sampling would be required to reveal any subtle differences among sites, and therefore, a conservative fishery management approach would assume the presence of population structure as suggested by the behavioral data and weakly supported by the current genetic data. If populations are divergent due to their evolutionary histories (“passive” structure), management strategies are best oriented to preserve the current level of genetic diversity in the lake. Smallmouth bass populations in Lake Erie have not been stocked or enhanced (K. Kayle, pers. comm.); therefore, admixture of stock and natural populations has not been a confounding factor. If populations are divergent because of biological and behaviorally based factors (“active” structure), not only should stocks remain unmixed, but also the factors leading to divergence should be managed. For smallmouth bass specifically, if divergence is due to nest fidelity, then management tactics could include the regional conservation and preservation of spawning sites.

Not only do these results support the use of multiple genetic data sources (Ruzzante *et al.* 1999), but also joint analyses of molecular and empirical field data are complementary for elucidating features of population structure. We continue to sample throughout the Great Lakes in order to address the implications of this question more thoroughly for smallmouth bass fishery management.

ACKNOWLEDGMENTS

We thank the following state, provincial, and university contacts for providing samples: Ontario Ministry of Natural Resources—Lake Erie Management Unit (L. Witzel, A. Cook, E. Arnold, E. Wright); New York State Department of Environmental Conservation (D. Einhouse and staff); Ohio Division of Wildlife, Ohio Department of Natural Resources—Sandusky Unit (R. Knight, J. Tyson, G. Emond, and staff), and Fairport Harbor Unit (K. Kayle, J. Deller, C. Knight, T. Bader); and Ohio State University (R. Stein, G. Steinhart, and associates). We thank M. Blum for sequencing samples at the CSU DNA Analysis Facility and students at Cleveland State University (T. Bowens, A. Ford), as well as Great Lakes Genetics Lab technicians C. Taylor (CSU) and D. Murphy (University of Toledo) for laboratory support. Financial support was provided by NOAA Ohio Sea Grant Project Number R/LR-5 award to CAS, and by a CSU Doctoral Dissertation Research Expense Award; Department of Biological, Geological, and Environmental Sciences (CSU) Travel Awards, and a grant from the CSU DNA Analysis Facility to WCB. Thoughtful and constructive critiques of the manuscript were provided by M. Coburn (John Carroll University), P. Doerder and R. Krebs (Cleveland State University), K. Kayle (Ohio Division of Wildlife), R. Strange (University of Toledo), A.A. Echelle (Oklahoma State University), and two anonymous reviewers for JGLR.

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Submitted: 22 July 2005

Accepted: 28 January 2006

Editorial handling: J. Ellen Marsden